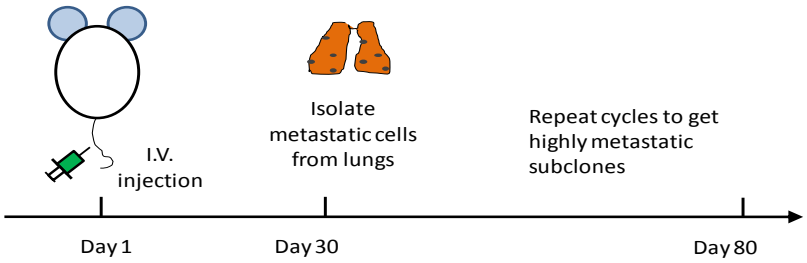
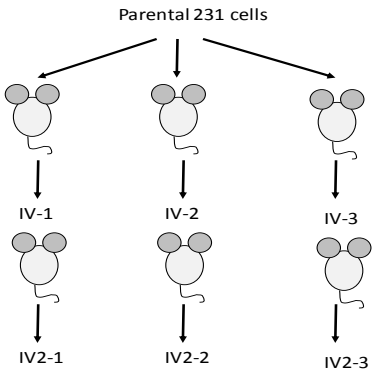
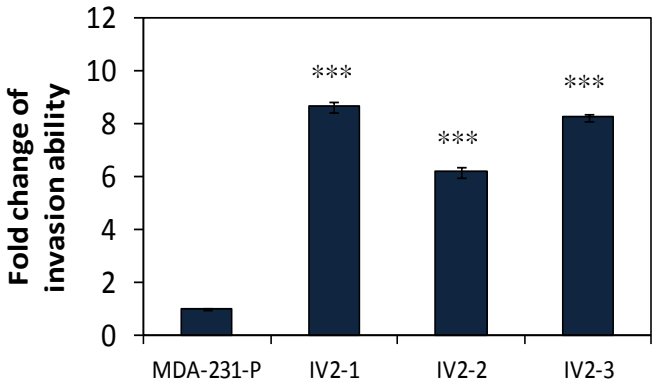


Breast cancer lines to be investigated

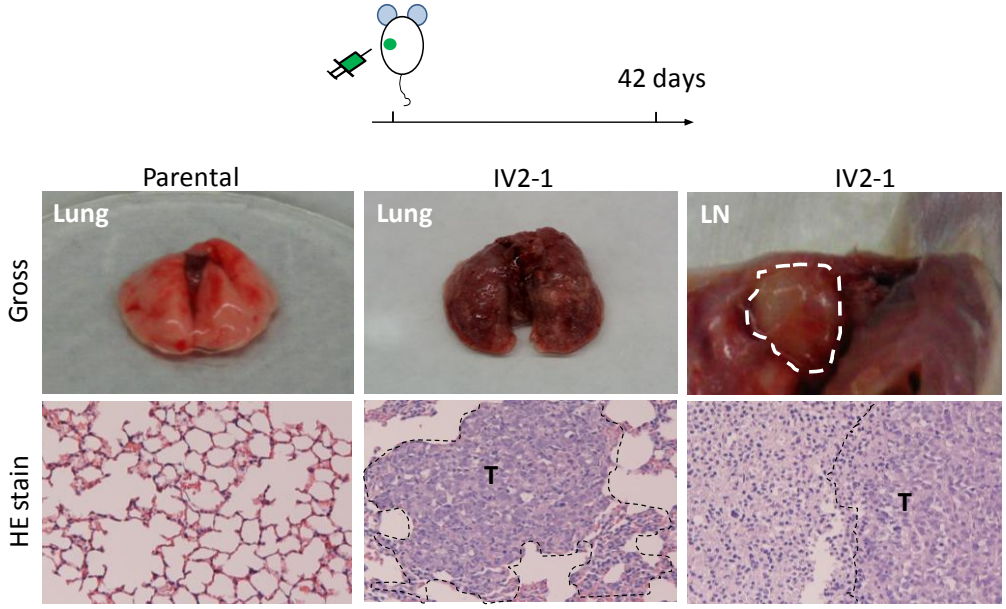
Cell line	In vitro phenotype	Cell number injected
MCF-7	Noninvasive	1×10^6
T-47D	Noninvasive	1×10^6
MDA-MB-453	Noninvasive	1×10^6
SK-BR-3	Noninvasive	1×10^6
BT-549	Invasive	1×10^6
Hs-578T	Invasive	1×10^6
MDA-MB-231	Invasive	1×10^6



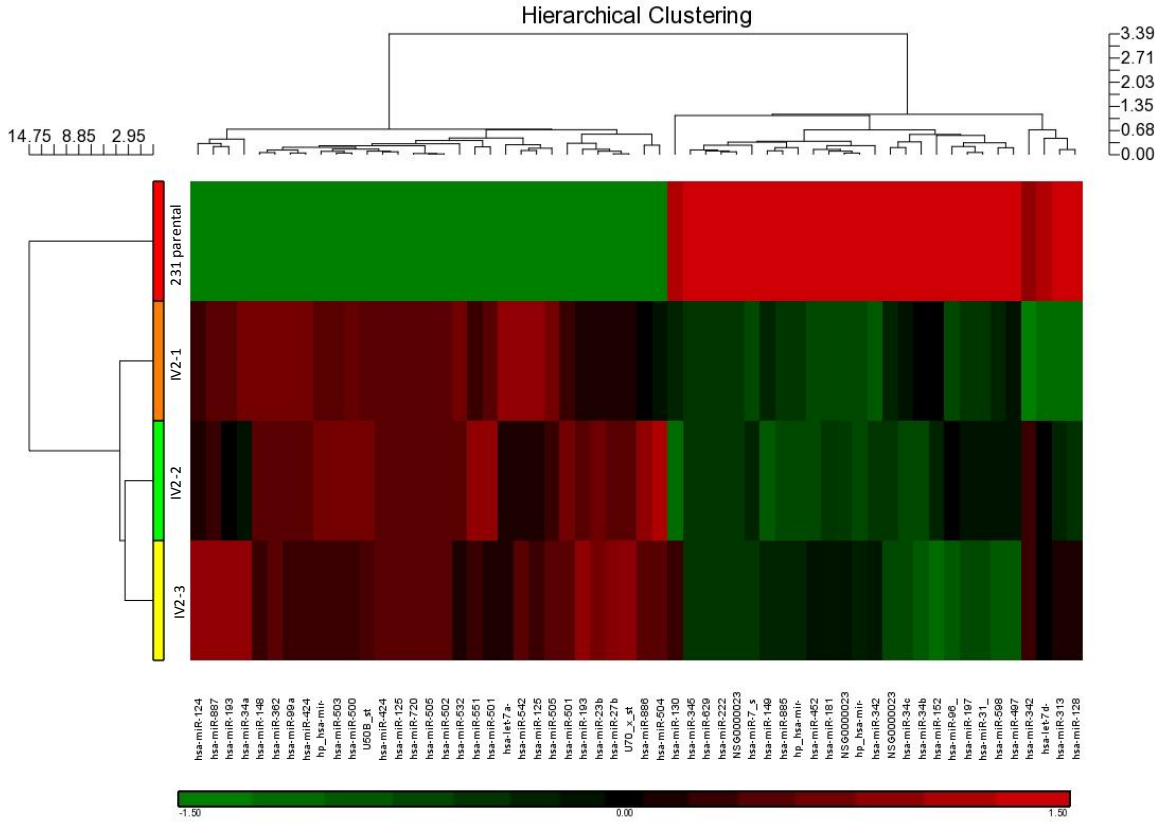
Supplementary Figure 1. Schematics for the establishment of highly metastatic breast cancer cells using a mouse model. First round selection: one million cells from each of breast cancer cell lines injected via tail veins into SCID mice and metastatic cells were isolated from lung 30 days post-injection. Second round selection: One million cells of the isolated metastatic IV cells were similarly injected and the lung metastatic cells were again recovered and amplified as above. The resulting IV2-1, IV2-2, IV2-3 cells derived from parental MDA-MB-231 were established as depicted.



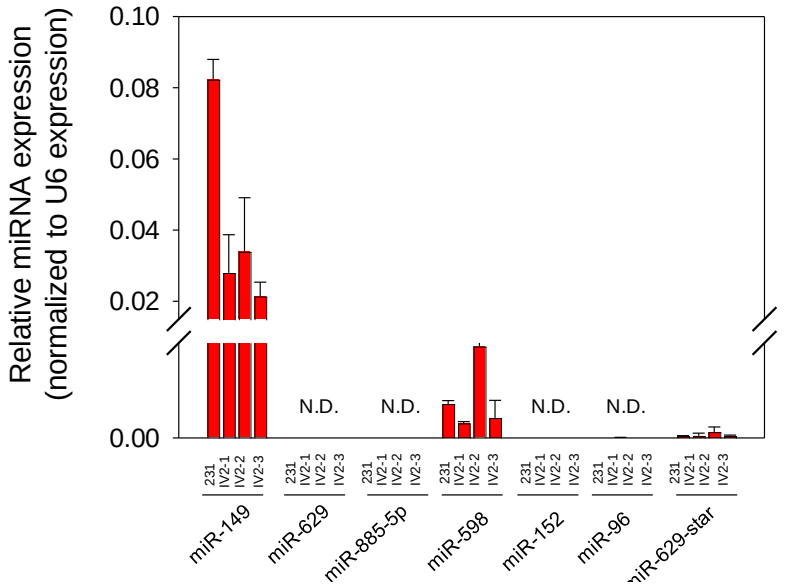
Supplementary Figure 2. Metastatic IV2 sublines exhibited increased invasive ability as compared to the parental 231 cells. Invasion ability of cells was measured using Biocoat invasion chamber (BD Biosciences) and determined by counting the number of cells invaded through matrigel-coated 8.0 μm well. All experiments were done in triplicate samples and repeated three times. *** $P<0.001$. All experiments were done in triplicate samples and repeated three times.



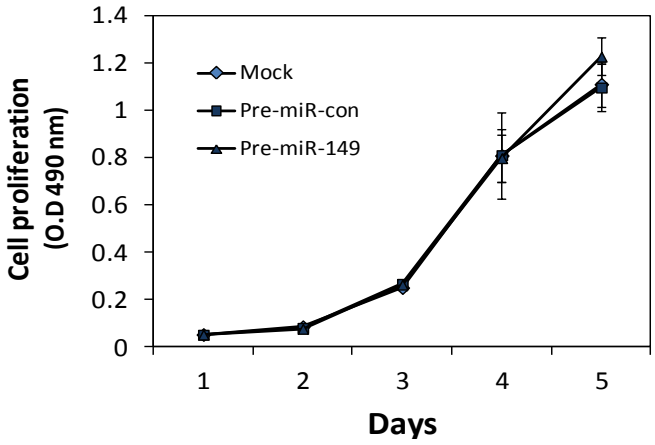
Supplementary Figure 3. Whole organ photographs and histological analysis of lung metastases generated by parental 231 and IV2-1 cells. Parental 231 cells and IV2-1 cells were injected in mice via tail vein. Lung and lymph nodes were examined for metastasis event by H&E staining 42 days after the injection. IV2-1 cells showed more aggressive lung and lymph node (LN) metastases as compared to the parental 231 cells. T = tumor.



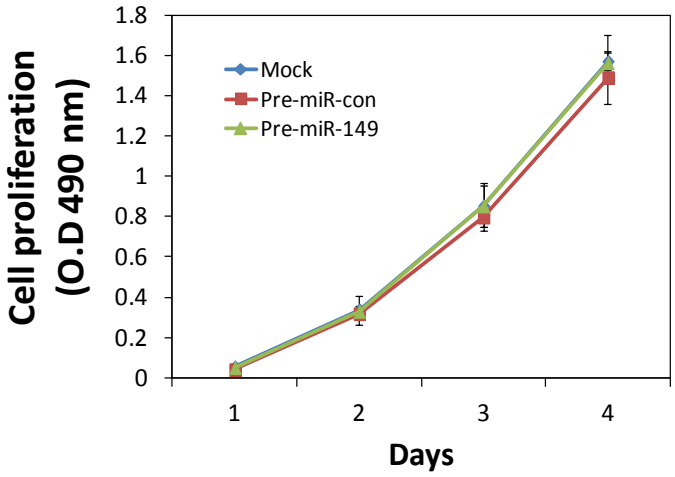
Supplementary Figure 4. Hierarchical clustering of miRNA expression between the parental 231 cells and three metastatic IV2 sublines, IV2-1, IV2-2 and IV2-3, revealed a set of differentially expressed miRNAs.



Supplementary Figure 5. Taqman qRT-PCR validation of selected candidate miRNAs. The level of miR-149, miR-629, miR-885-5p, miR-598, miR-152, miR-96 and miR-629-star were analyzed in parental 231 cells and three IV2 sublines using Taqman qRT-PCR. All experiments were done in triplicates and repeated three times. N.D. represents" not detected".

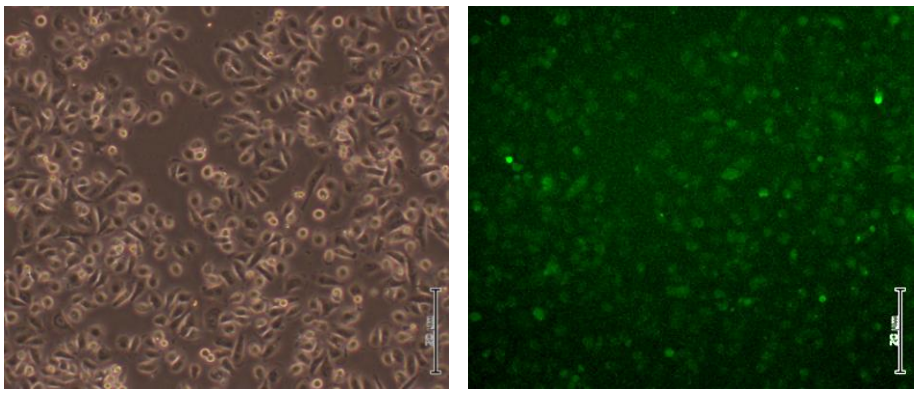


Supplementary Figure 6. Overexpression of pre-miR-149 did not affect proliferation of the IV2-1 cells. 3000 cells were seeded on each chamber of a 96-well plate and analyzed at the indicated time point after initial seeding. CellTiter 96 AQueous One Solution (Promega, Madison, WI, USA) was used according to manufacturer's instruction. All experiments were done in triplicates and repeated three times.

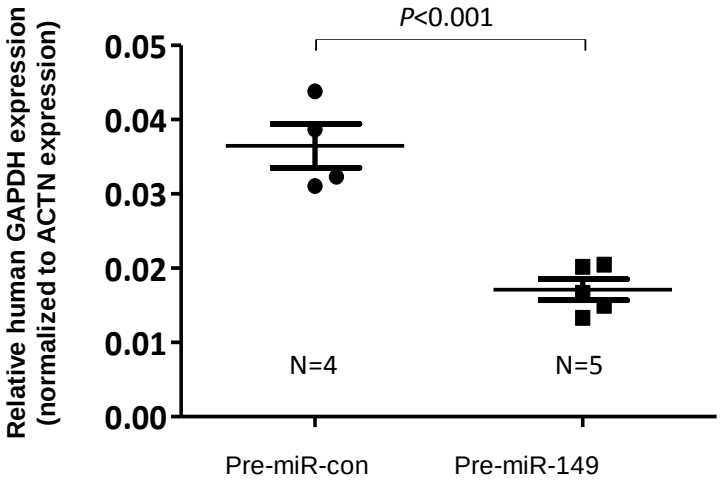


Supplementary Figure 7. Overexpression of pre-miR-149 did not affect proliferation of the Hs578T breast cancer cells. All experiments were done in triplicates and repeated three times.

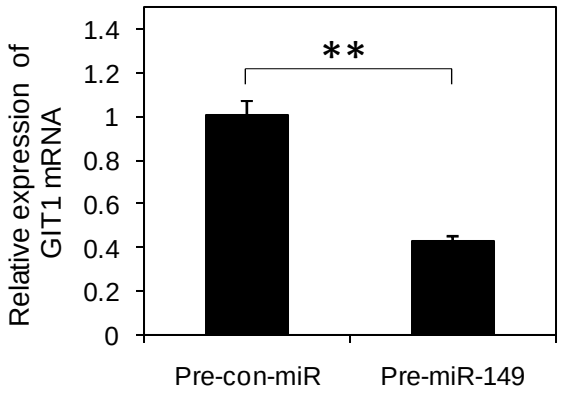
FAM-labeled Pre-miR-149 transfectioin



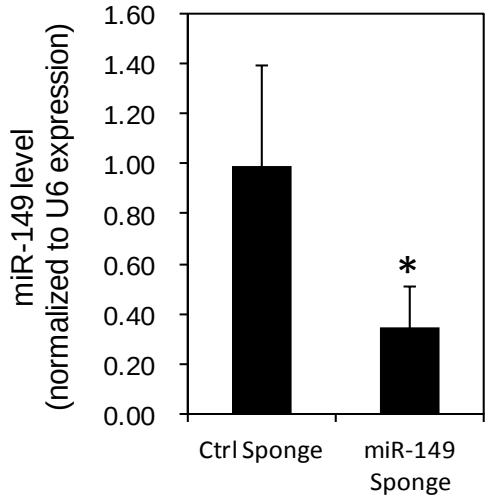
Supplementary Figure 8. Evaluation of transfection efficiency of pre-miR-149 using FAM-labeled pre-miR-149. Left, bright field, Right, dark field. IV2-1 Cells were transfected with FAM-labeled pre-miR-149 and transfection efficiency was measured after 24hr by fluorescence microscope. Over 90% of the transfected cells expressed green fluorescence, indicating high efficiency of the microRNA transfection. All experiments were repeated three times.



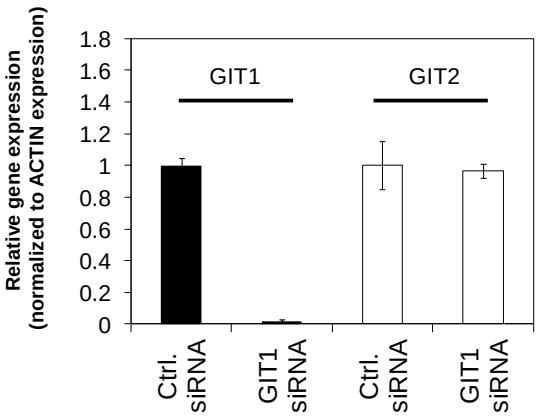
Supplementary Figure 9. miR-149 reduced lung targeting ability of metastatic IV2-1 cells. The control IV2-1 cells and pre-miR-149-transfected IV2-1 cells were injected into mice tail vein. After 24 hr, lung perfusion was performed and RNA of mouse lung was extracted. IV2 cells were detected by qRT-PCR using human-specific GAPDH primer. Human GAPDH expression in lung from 4 mice in the control group and 5 mice in the pre-miR-149-transfected group was shown.



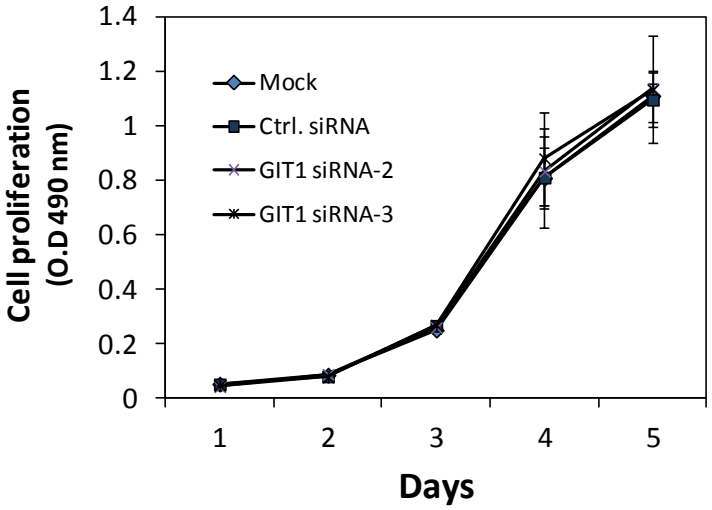
**Supplementary Figure 10. miR-149 suppressed GIT1 expression at the mRNA level in IV2-1 cells. **** $P < 0.01$. All experiments were done in triplicates and repeated three times.



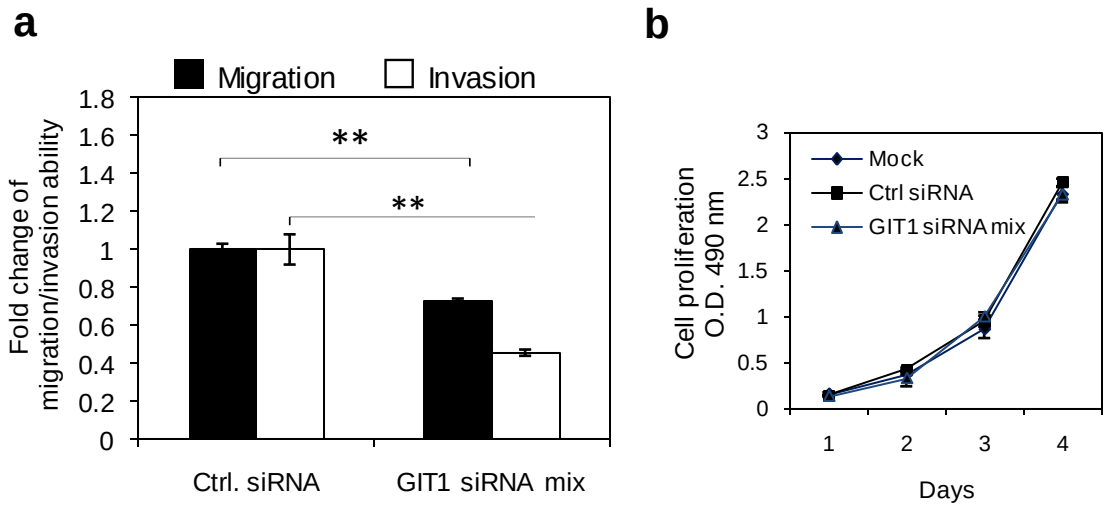
Supplementary Figure 11. miR-149 sponge suppressed miR-149 expression in parental 231 cells. ***P*<0.05. All experiments were done in triplicates and repeated three times.



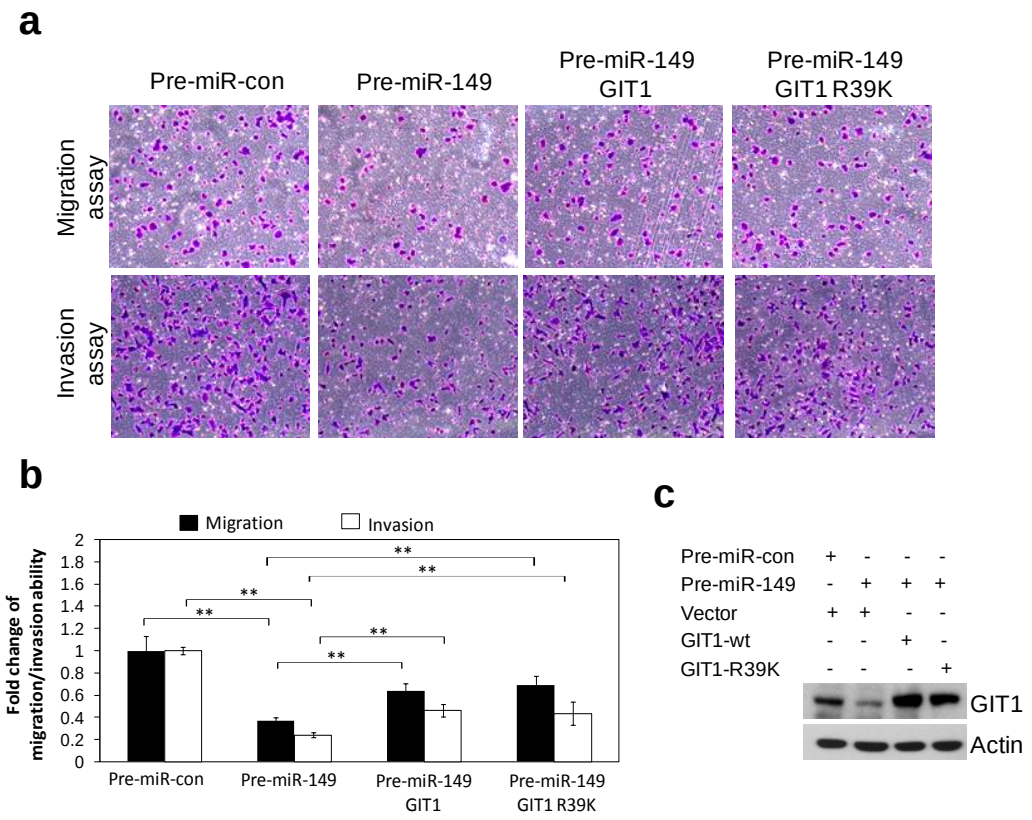
Supplementary Figure 12. Knockdown of GIT1 using siRNA transfection did not affect GIT2 mRNA expression. The experiment was done in triplicates and repeated three times.



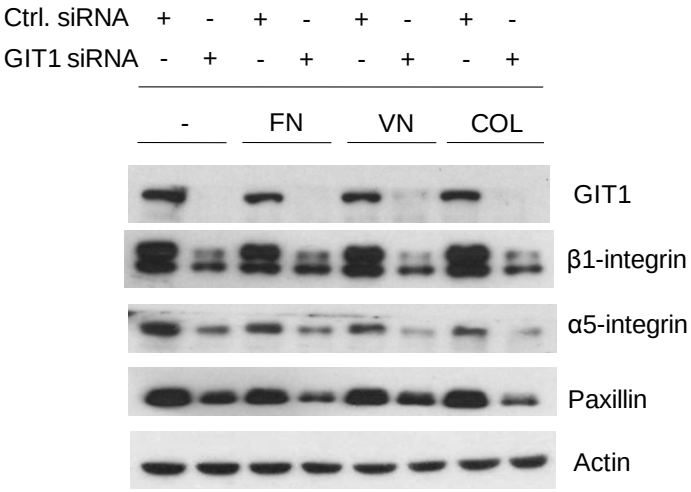
Supplementary Figure 13. Depletion of GIT1 did not affect proliferation of the IV2-1 cells. All experiments were done in triplicates and repeated three times.



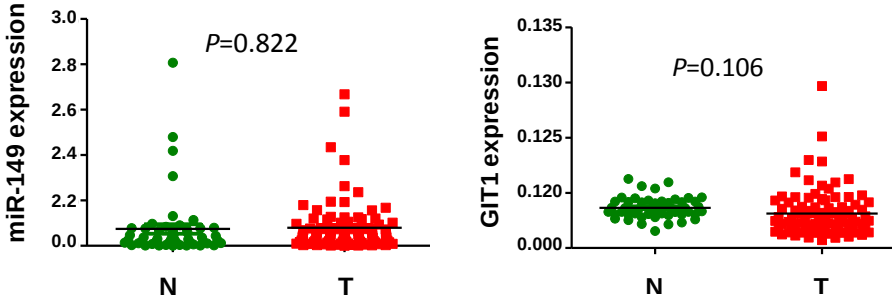
Supplementary Figure 14. Inhibition of GIT1 in the invasive Hs578T cells reduced their migration and invasion. (a) Depletion of GIT1 suppressed migration and invasion of the cells. **(b)** Depletion of GIT1 did not affect proliferation of the cells. ****** $P < 0.01$. All experiments were done in triplicates and repeated three times.



Supplementary Figure 15. ArfGAP activity of GIT1 is not required for GIT1-mediated cell migration and invasion of IV2 cells. (a,b) Analysis of the effect of overexpression of R39K mutant GIT1 and wild type GIT1 on pre-miR-149 transfected IV2-1 cells using Boyden chamber migration/invasion assay. Representative photographs of the migrated/invaded cells from different treatments are shown. Quantitative data are shown by histograms. **(c)** Western blotting analysis of overexpression of wild type GIT1 and R39K mutant GIT1 in pre-miR-149-transfected IV2-1 cells. ****** $P< 0.01$. All experiments were done in triplicates and repeated three times.



Supplementary Figure 16. Effect of depletion of GIT1 on paxillin and α5β1 integrin level under different matrices-coating conditions. 5 x10^5 cells were seeded on fibronectin (FN)- and vitronectin (VN)- and type IV collagen (COL)-coated 3.5-cm dishes. Cells were harvested and protein was extracted using RIPA lysis buffer 6 hours after seeding. Protein extracts were subjected to Western blotting analysis. All experiments were repeated three times.



Supplementary Figure 17. Expression level of miR-149 and GIT1 in 90 breast tumor specimens and 50 adjacent normal tissues as determined by qRT-PCR.

Supplementary Table 1. Metastasis ability between parental 231 cells and IV2-1 cells.

	Lung metastasis	Lymph node metastasis
MDA-231-P	0/5 (0%)	0/5 (0%)
IV2-1	5/5 (100%)	3/5 (60%)

Supplementary Table 2. Candidate miRNAs down-regulated in IV2 lines.

Probeset ID	Fold-Change (IV2-1 vs. 231-P)	Fold-Change (IV2-2 vs. 231-P)	Fold-Change (IV2-3 vs. 231-P)	p-value	Average of fold-change among IV2 lines
hsa-miR-629-star	-6.49114	-6.04192	-6.35662	8.99E-08	-6.29656
hsa-miR-885-5p	-4.69435	-5.63412	-7.30055	4.58E-08	-5.87634
hsa-miR-152	-3.03444	-7.60293	-3.68091	2.55E-07	-4.77276
hsa-miR-149	-3.82555	-3.86429	-5.20837	1.10E-07	-4.299403333
hsa-miR-7	-4.82596	-3.91011	-3.11753	9.25E-08	-3.9512
hsa-miR-598	-2.5022	-5.70723	-3.52322	4.18E-08	-3.910883333
hsa-miR-34b-star	-2.5714	-3.94694	-3.9894	5.38E-08	-3.50258
hsa-miR-96	-3.66498	-3.82845	-2.95947	7.67E-08	-3.4843
hsa-miR-31	-3.18798	-3.73363	-2.45634	7.74E-08	-3.125983333

Supplementary Table 3. The list of primers and oligomers used in this study.

Oligomers	Sequences
U6 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACAAAAATAT-3’
miR-149 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACGGGAGT-3’
U6 forward primer	5’-TTCCTCCGCAAGGATGACACGC-3’
miR-149 forward primer	5’-GGTCTGGCTCCGTGTCTTC-3’
Universal reverse primer	5’-GCTGACTCCTAGTCC-3
miR-629 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACAGTTCT
miR-629 forward primer	5’-TGGGTTTACGTTGGG
miR-629-star RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACGCTGGG
miR-629-star forward primer	5’-GTTCTCCCAACGTAAG
miR-885-5p RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACAGAGGC
miR-885-5p forward primer	5’-TCCATTACACTACCCT
miR-152 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACCCAAGT
miR-152 forward primer	5’-TCAGTGCATGACAGA
miR-96 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACAGCAAA
miR-96 forward primer	5’-TTTGGCACTAGCACATT
miR-598 RT primer	5’-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCAC CAGAGCCAACTGACGA
miR-598 forward primer	5’-TACGTCATCGTTGTCA

Supplementary Table 3 continued. The list of primers and oligomers used in this study.

Oligomers	Sequences
Human ACTIN forward primer	5’- CGGCA TCGTC ACCAACTG
Human ACTIN reverse primer	5’- TCTCA AACAT GATCTGGGTC ATCT
GIT1 forward primer	5’-GCCGACAGTGACTATGAGAA
GIT1 reverse primer	5’-AGTTCCTGAATGTTCTTGGT
GIT2 forward primer	5’-ACCTCAAATGGCAGACAGCA
GIT2 reverse primer	5’-CGTCTCTCGCCTGTCAACTT
Human GAPDH forward primer	5’-GGTCACCAGGGCTGCTTTTA
Human GAPDH reverse primer	5’-TTCCCGTTCTCAGCCTTGAC
miR-149 Sponge-forward oligonucleotide	5’-TCGAGGGGAGTGAAGCACGGAGCCAGAACTAGG GAGTGAAGCACGGAGCCAGAACTAGGGAGTGAAGC ACGGAGCCAGAACTAGGGAGTGAAGCACGGAGCCA GAACTAGGGAGTGAAGCACGGAGCCAGAACTAGGG AGTGAAGCACGGAGCCAGAACTAGGGAGTGAAGCA CGGAGCCAGAGGTAC-3’
miR-149 Sponge-reverse oligonucleotide	CTCTGGCTCCGTGCTTCACTCCCTAGTTCTGGCTCCG TGCTTCACTCCCTAGTTCTGGCTCCGTGCTTCACTCC CTAGTTCTGGCTCCGTGCTTCACTCCCTAGTTCTGGC TCCGTGCTTCACTCCCTAGTTCTGGCTCCGTGCTTCA CTCCCTAGTTCTGGCTCCGTGCTTCACTCCCC.
Control Sponge-forward oligonucleotide	5’-TCGAGAAATGTACTGCGCGTGGAGACGACTAAAA TGTACTGCGCGTGGAGACGACTAAAATGTACTGCGC GTGGAGACGACTAAAATGTACTGCGCGTGGAGACG ACTAAAATGTACTGCGCGTGGAGACGGGTAC-3’
Control Sponge-reverse oligonucleotide	5’-CCGTCTCCACGCGCAGTACATTTTAGTCGTCTCCA CGCGCAGTACATTTGGTAGTCGTCTCCACGCGCAGT ACATTTTAGTCGTCTCCACGCGCAGTACATTTTAGTC GTCTCCACGCGCAGTACATTTC-3’
GIT1-R39K-forward primer	TGCTGCAGCGTGCACAAGAGCCTGGGACGCCAC
GIT1-R39K-reverse primer	GTGGCGTCCCAGGCTCTTGTGCACGCTGCAGCA

GIT1-FL-forward primer	5’-GGCCGAATTCGGATGTCCCGAAAGGGGCC
GIT1-FL –reverse primer	5’-GGCCGGTACCTCACTGCTTCTTCTCTCG
GIT1-3’UTR-forward primer	5’-GCTCTAGAGCATCACCACCCGAGAGAA
GIT1-3’UTR-reverse primer	5’-GGCCGGCCTCTTGACAGCCCACCCACCA
GIT1-mutant 3’UTR-forward primer	5’-GCGCCCCACCCCCATTCGAACTTTCAGCCCTACTGG
GIT1-mutant 3’UTR-reverse primer	5’-CCAGTAGGGCTGAAAGTTCGAATGGGGGTGGGGCGC
Negative control siRNA	Sense: 5’-UUCUCCGAACGUGUCACGUTT-3’ Anti-sense: 5’-ACGUGACACGUUCGGAGAATT-3’
GIT1 siRNA-1	Sense: 5’-CGAGCUGCUUGUAGUGUAUTT-3’ Anti-sense: 5’-AUACACUACAAGCAGCUCGTT-3’
GIT1 siRNA-2	Sense: 5’-GUGCCAAUAUGAGCUCAGUTT-3’ Anti-sense: 5’-AGUGAGCUCAUAUUGGCACTT-3’
GIT1 siRNA-3	Sense: 5’-CCUUGAUCAUCGACAUUCUTT-3’ Anti-sense: 5’-AGAAUGUCGAUGAUCAAGGTT-3’

Supplementary Table 4. Information of antibodies and reagents.

Western blotting antibodies	
	Anti-GIT1 (BD Biosciences)
	Anti-phospho-Paxillin (Cell Signaling Technology)
	Anti-Integrin β1 (Santa Cruz Biotechnology)
	Anti-FAK (Millipore)
	Anti-Integrin α5 (Signaling Technology)
	Anti-Paxillin (Millipore)
	Anti-phospho-Fak Tyr861 (Millipore)
	Anti-phospho-Fak Tyr 397 (Abcam)
Immunohistochemisry	

antibody	
	Anti-GIT1 (Bethyl laboritories, Inc.)
Immunostaining antibodies	
	Anti-Vinculin (Sigma-Aldrich)
	Alexa Fluor 488 anti-mouse (Invitrogene)
Chemicals	
	Bafilomycin A1 (R&D systems)
	Cyclohexamide, MG132, Blasticidin (Sigma-Aldrich)
	Ammonia chloride (NH_4Cl), Fibronectin, type IV collagen and vitronectin (Merck & Co., Inc.)
	Fibronectin, vitronectin, Type IV collage (Millipore)